J. Anat. (1969), **105**, 2, pp. 295–306
With 8 figures
Printed in Great Britian

Species differences in internode formation following two types of peripheral nerve injury

JEAN M. JACOBS AND J. B. CAVANAGH
*M.R.C. Research Group in Applied Neurobiology, Institute of Neurology, Queen Square, London, W.C.*1

(*Received* 31 *October* 1968)

INTRODUCTION

The wide range of internodal lengths and diameters found in fibres of normal peripheral nerves is a reflexion of the different times at which the fibres begin to myelinate. The first fibres to myelinate are destined to have the longest internodes because they are subjected to the lengthening process caused by growth of the part for the longest time; in general, these fibres will also tend to be the thickest.

However, when nerve fibres are regenerating after a crush injury in an adult animal that has ceased growth, they are not subjected to this extension in length. Such regenerated internodes were found by several workers to be of approximately uniform length. Hiscoe (1947) in rats and Vizoso & Young (1948) using rabbits found that all internodes were of the order of 300 μm. More recently Fullerton, Gilliatt, Lascelles & Morgan-Hughes (1965) observed regenerated internodes in guinea-pigs to be about 400 μm in length.

Numerous experiments (Sanders & Young, 1944; Simpson & Young, 1945; Hillarp & Olivecrona, 1946; Aitken, Sharman & Young, 1947; Aitken & Thomas, 1962) have been devised to show that following nerve regeneration, the ultimate fibre diameter is influenced both by peripheral connexions finally established and by some factor in the proximal stump. Influences controlling internodal length, however, have not been fully investigated.

It has been assumed that the internodes of 300–400 μm found in regenerated adult nerves after a crush injury represent the 'basic' length of the Schwann cell; that is the length of the Schwann cell at the commencement of myelination during normal development. Evidence from light microscope studies has suggested that myelin does not begin to form until the axon has attained a diameter of 1 μm (Duncan, 1934; Vizoso & Young, 1948). An electron microscope study (Gamble, 1966) has shown that myelination in humans may begin when axons are only 0·7 μm in diameter; Allt (1969) similarly found that rat nerve fibres began to myelinate when 0·5 μm in diameter. If axon thickness is a critical factor in determining the time at which myelination commences, it was considered that it might also exert some influence on other aspects of myelination.

It has been found that diphtheria toxin in many species damages Schwann cells in a specific manner; when injected intraneurally in the appropriate dose the toxin can cause total loss of myelin sheaths within a restricted region of the injected nerve in

chickens and rats (Jacobs, Cavanagh & Mellick, 1966; Jacobs, 1967). Although the myelin-bearing function of the Schwann cells is temporarily impaired by this process, they do not lose their viability and are still quite capable of proliferation. Such a process provides, therefore, an interesting system in which to study remyelination, and one that is complementary to regeneration after nerve crush, since the axons remain of normal thickness. After nerve crush, new myelin sheaths are found within a few days of the regrowth of axons into the degenerated peripheral stump of the nerve (Quilliam, 1958), and at this stage, the axons are of uniformly small diameter.

Thus, these two types of degeneration provide quantitatively different circumstances each of which could influence the Schwann cell differently in its function of forming new myelin sheaths.

The purpose of this study was, therefore, to compare the formation of new internodes after a nerve crush and following a purely demyelinating lesion. Two species were studied and an unexpected difference in the pattern of internode formation in each was revealed.

MATERIALS AND TECHNIQUES

Animals. The chickens were males of Rhode Island Red × Silver Link strain. Those used for intraneural injection weighed 1270–1500 g and were aged about 6 months; within this weight range, limb growth was virtually ceased (Matheson, 1968). Birds used for the nerve crush experiments were fully adult and weighed 2125–2180 g. The chickens were housed singly in cages and fed with Battery pellets.

Rats were adult males of WAG/C strain, weighing 420–450 g. They were housed in plastic cages and fed on *Chardex*.

Toxin. Toxin was kindly provided by Dr Mollie Barr, formerly of the Wellcome Research Laboratories, Beckenham. 1 ml contained 40 guinea-pig L^+ doses. The toxin was used without added antitoxin. The use of the notation L^+ as a measure of toxicity is strictly incorrect when toxin is used without antitoxin, but it was been retained for convenience. The amended definition of an L^+, therefore, becomes 'that amount of toxin which *if combined* with 1 unit of antitoxin would kill a 250 g. guinea-pig in 5 days.' This quantity of toxin has about thirteen times the toxicity of a correctly defined L^+ dose, when assessed by neurotoxic effect (Jacobs *et al.* 1966).

The toxin was diluted in sterile normal saline; trypan blue was added to colour the solution giving a concentration of 2 μg in 0·01 ml toxin solution.

Intraneural injection of toxin. Details of the injection procedure are described in previous papers (Jacobs *et al.* 1966; Jacobs, 1967). In both chickens and rats, the sciatic nerve was exposed under Nembutal and ether anaesthesia, and 0·01 ml toxin solution was injected with a fine needle and an Agla micrometer syringe. The injection was directed towards the body; in chickens the coloured solution ran rapidly in a proximal direction for 1–2 cm and distally for a few mm from the point of entry of the needle; in the rats the upper limit of the dyed toxin solution usually passed beyond the field of view. The dose of toxin found to produce total demyelination within the injected region of the nerve was $1{\cdot}25 \times 10^{-5}$ L^+ for chickens and $2{\cdot}5 \times 10^{-2}$ L^+ for rats.

Crushing of chicken sciatic nerve. The sciatic nerve was exposed under Nembutal

and ether anaesthesia, and was crushed for 30 sec with fine smooth-tipped watchmakers' forceps at a level of about 1 cm distal to the sciatic notch.

Treatment of tissues. Chickens were killed by an overdose of Nembutal injected intravenously. Rats were killed by excess of ether. In this work (forming part of study on the reactions of Schwann cells) it was important that cell nuclei should be clearly visible, and therefore, the technique of Helly fixation followed by staining with Oil Red 4B and haemalum was developed.

The sciatic nerve from the sciatic notch to the lower end of the femur was quickly removed and laid on card under slight tension. When the nerve was adherent to the card, it was placed in Helly's fiuid for 24–48 h. After washing in water a segment of the nerve was removed for examination; in injected nerves, this was about 0·5–1·5 cm above the site of injection. In crushed nerves, a segment was taken *c.* 2·0–3·0 cm distal to the point of crushing. The epineurium and perineurium were removed and the remaining bundle of fibres was slightly separated to allow solutions to penetrate. The mass of fibres was treated with iodine and sodium thiosulphate to remove mercuric chloride deposit and, after washing thoroughly, was stained with OR4B in 60 % isopropyl alcohol for 30–45 min and rinsed in 60 % isopropyl alcohol to remove excess dye. After washing in water, the fibres were counter-stained with Mayer's haemalum for about 7 min and then 'blued' in tap water.

Final teasing of fibres was carried out in a drop of 33 % glycerine under a dissecting microscope with mounted sewing needles (gauge 12); the preparations were mounted in 33 % glycerine under circular cover-slips and ringed with asphalt varnish.

Measurements of various parameters of nerve fibres in teased preparations. External diameters and axon diameters were measured with ×12·5 movable ocular micrometer in conjunction with a × 57 or × 100 oil-immersion objective. Three measurements of each parameter were made along each internode at points other than the paranodes and the nuclear region, where variations in diameter are expected to occur. The mean of the three readings was derived. Internodal lengths were measured from a tracing drawn by projecting the image from the microscope on to paper in a darkened room. The magnification factor was measured from the image of a micrometer slide projected under similar conditions. It was usually possible to project a complete internode in one field of the microscope, but for very long internodes it was necessary to draw the whole internode in two parts. Three readings of the length of each internode were made, using a measuring wheel calibrated in cm. Readings were made to the nearest 0·2 cm and the mean length in μm derived.

Effects of fixation and staining upon nerves. Distortions are introduced during the fixation, staining and mounting of nerve fibres. They have been investigated and discussed by Williams & Wendell-Smith (1958), Williams (1959), and Wendell-Smith & Williams (1959). It has not been found possible to assess these errors exactly in our material. No satisfactory method exists for assessing the amount of shrinkage produced during fixation of nerve fibres. The separate constituents of Helly's fluid produce only slight change in the volume of whole livers (Baker, 1960), but their effect upon nerves is not known. During staining, dehydration in alcohols is known to cause considerable shrinkage, but since the fibres were only subjected for a short time to 60 % isopropyl alcohol and then rehydrated, this is considered unlikely to cause appreciable changes in the dimensions of nerve fibres.

Williams & Wendell-Smith (1958) have shown that when fibres are mounted in a solution whose refractive index differs markedly from that of the myelin sheath, the optical properties of the highly refractile myelin sheath lead to a distortion of the true measurement of the axon diameter. Estimates of the ratio of axon diameter to external nerve diameter were made in normal chicken nerve fibre mounted in 33 % glycerine. A mean value of 0·49:1 was obtained for fibres of 10–12 μm diameter. This compares reasonably well with a ratio of 0·51:1 obtained by Williams & Wendell-Smith from measurements of thin transverse sections of unfixed mammalian nerve fibres in the range 10–12 μm.

It appears, then, that the various errors introduced during fixation and staining and those due to the optical properties of the myelin sheath result in relative dimensions of axon diameter to myelin sheath thickness that do not differ markedly from those in fresh fibres, although the total shrinkage of the fibres is not known.

RESULTS

Internodal lengths in chickens on recovery from demyelination caused by diphtheria toxin

Eight chickens were injected intraneurally with $1{\cdot}25 \times 10^{-5}$ L^+ diphtheria toxin. Birds were killed on the 12th, 18th and 22nd d following the injection to confirm that there was complete demyelination of all fibres within the affected region with this dose. By the 14th or 15th d, the injected limbs had all become paralysed. At 12 d, all myelinated fibres were showing signs of disintegration, and by the 18th d only myelin debris remained, mainly in large fibres. Twenty-two days after injection scarcely any myelin debris remained and new myelin was forming. Every fibre was affected; a normal myelinated fibre would be very conspicuous among the mass of pale demyelinated or newly remyelinating fibres.

The remaining animals were killed at intervals of 103, 206 and 307 d after injection. External nerve diameters, axon diameters and internodal lengths were measured. Similar measurements were also made on fibres from the same region of the normal uninjected bundle of the animal killed at 307 d. A graph expressing internodal length against axon diameter (Fig. 1) shows that there is a linear relationship between these two parameters and that the internodal lengths of the largest fibres exceed 2000 μm.

The results of measurements on the remyelinating animals are shown in Figs. 2–4. Internodal lengths are plotted (in the upper graphs) against axon diameters, in preference to the more usual external diameter, in order that direct comparisons might be made between remyelinating and normal fibres. The lengths of internodes on each fibre were plotted against axon diameter and these points then joined up by a vertical line. This is a method suggested by Fullerton *et al.* (1965) to show the distribution of internodal lengths along individual fibres.

The mean internodal length was found for each axon diameter and a regression line was calculated from these figures (lower graph, Figs. 2–4). This procedure was taken from Vizoso & Young (1948). Regression coefficients, correlation coefficients and y-axis intercepts were calculated from fibres of experimental animals and from normal fibres (Table 1).

In Figs. 2, 103 d after injection of toxin, while there was a wide scatter of internodal lengths along the individual fibres, there appeared to be a positive correlation between the mean internodal lengths and the corresponding axon diameter; the thinnest fibres tending to have the shortest internodes and the thickest fibres tending to have the longest internodes. It will be observed that the complete spectrum of normal axon diameters is present, confirming that the axons were spared during the demyelinating process.

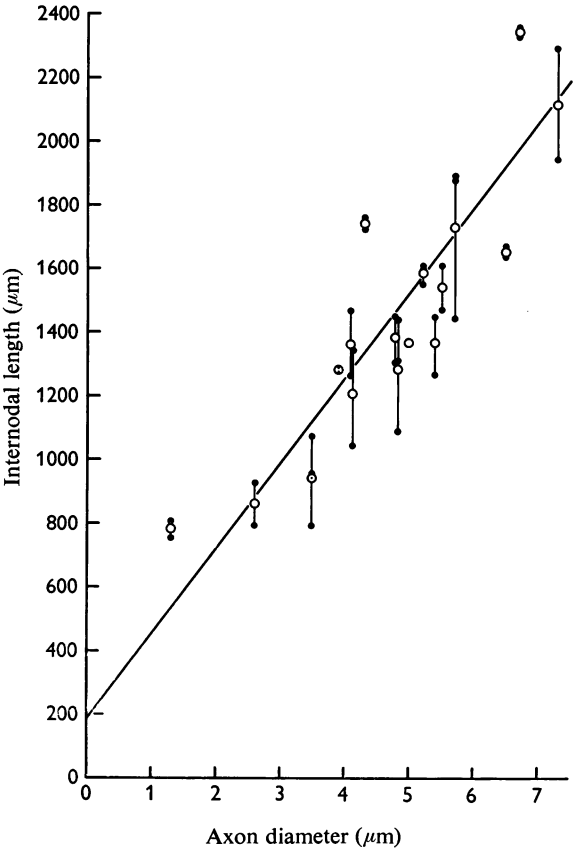

Fig. 1. Graph of internodal lengths (μm) and axon diameters (μm) in normal fibres from the sciatic nerve of a chicken. The open rings indicate the mean internodal lengths for individual fibres. The regression line was calculated from these mean values. Solid circles indicate limit of range of internodal lengths.

Figure 3 shows the values of internodal lengths and axon diameters 206 d after injection of toxin. The positive correlation between these two parameters is again obvious. At 307 d, there is still a persistence of this linear relationship (Fig. 4).

The slope of the regression line (regression coefficient) from the normal fibres is seen to be two or three times that of the remyelinating fibres at each time (Table 1). Moreover, the internodal lengths of the largest fibres reach a maximum of only about 1000 μm, whilst the internodal length of a normal fibre of equivalent calibre would be

over 2000 μm. Thus, one original internode tends to be replaced by two or three new internodes during regeneration, and the mean lengths of the internodes at each axon diameter do not alter once myelin formation is well established.

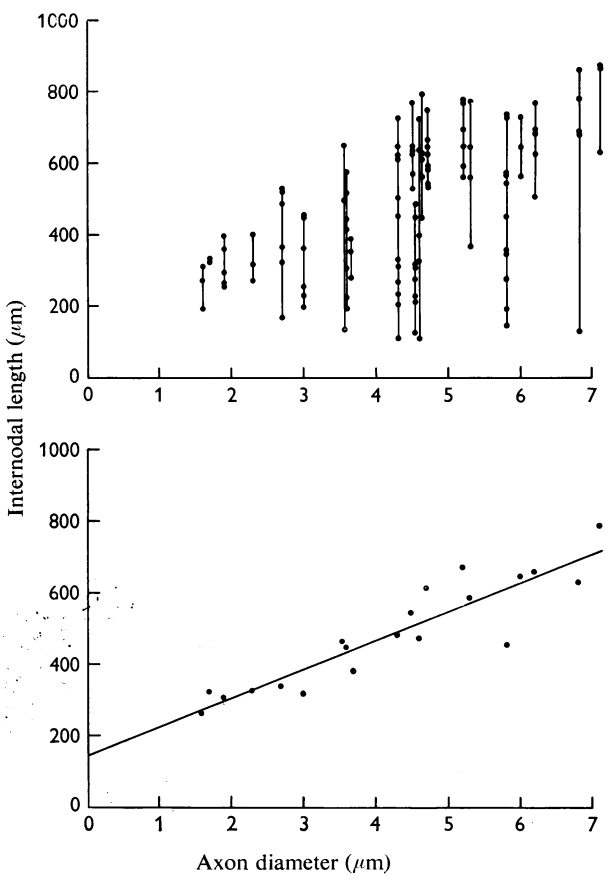

Fig. 2

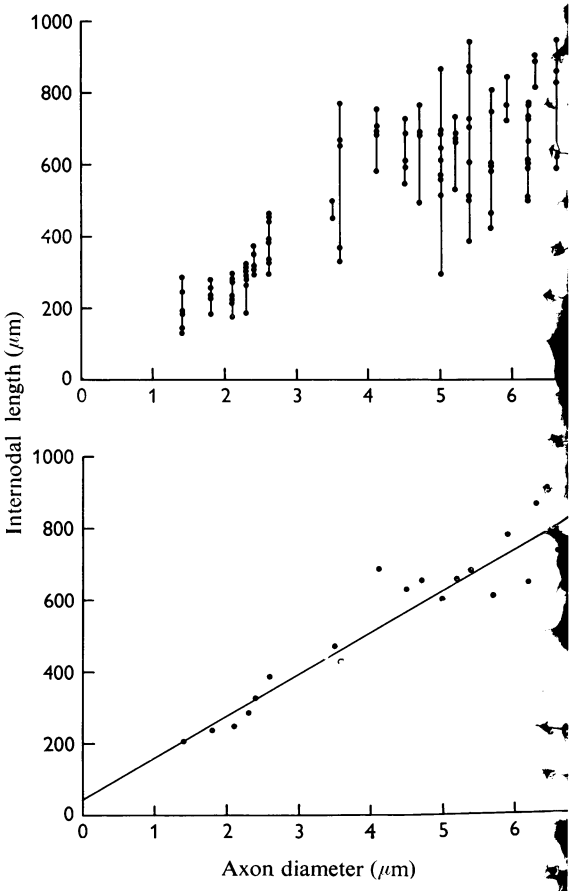

Fig. 3

Figs. 2–4. *Upper graphs*: internodal lengths (μm) and axon diameters (μm) of remyelinating nerve fibres from chickens injected intraneurally with diphtheria toxin 103 d (Fig. 2), 206 d (Fig. 3) and 307 d (Fig. 4) previously. The lines indicate individual nerve fibres, and the solid circles each internodal length. *Lower graphs*: relationship between *mean* internodal lengths and axon diameters from the same fibres. In this and subsequent graphs regression lines were calculated from the mean values of internodal lengths for each axon diameter.

Table 1. *Statistical data derived from results constituting Figs.* 1–4

Interval following injection of toxin	Regression coefficient	Correlation coefficient	y-axis intercepts
Normal animal	1·46	0·90	188
103 d	0·40	0·77	144
206 d	0·58	0·98	40
307 d	0·49	0·64	230

In both toxin-injected and crushed nerves, there was a wide scatter of remyelinating internodal length along individual fibres. There was no suggestion, in the toxin-injected nerves, that this variability was due to the presence of 'undemyelinated' internodes.

First, examination of nerve fibres 12, 18 and 22 d after injection confirmed that all fibres had been damaged by toxin.

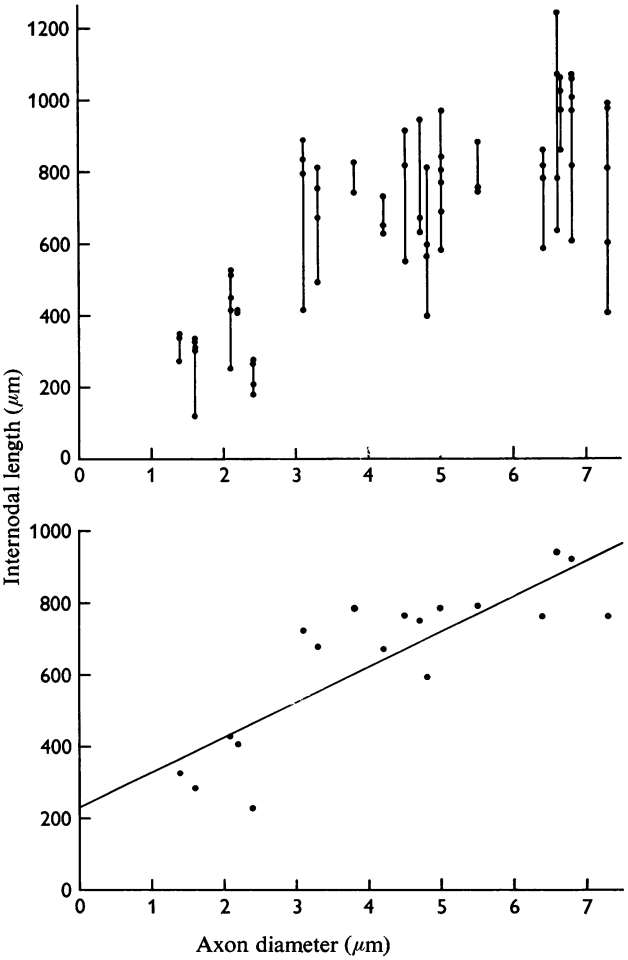

Fig. 4. For legend see facing page.

Secondly, although measurements of fibre diameters were not made for every internode along an individual fibre, it is clear from the tracings used to derive internodal lengths that there was no evident variation in diameter of each internode along individual fibres. If any original internodes had persisted they would have been easily identified in this way.

Thirdly, comparison of the graphs of normal fibres and fibres remyelinating after toxin injection confirms that no internodes of normal length remained among the remyelinating fibres.

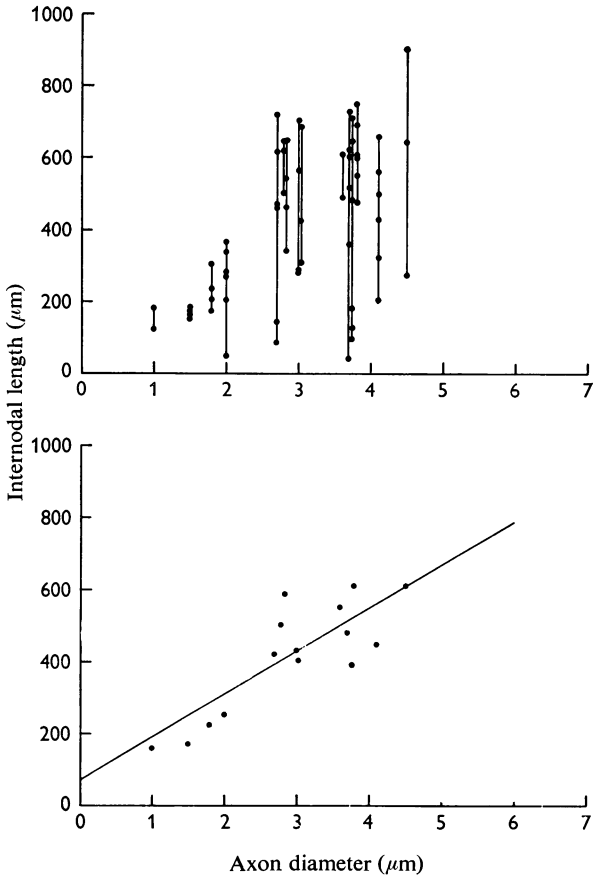

Fig. 5

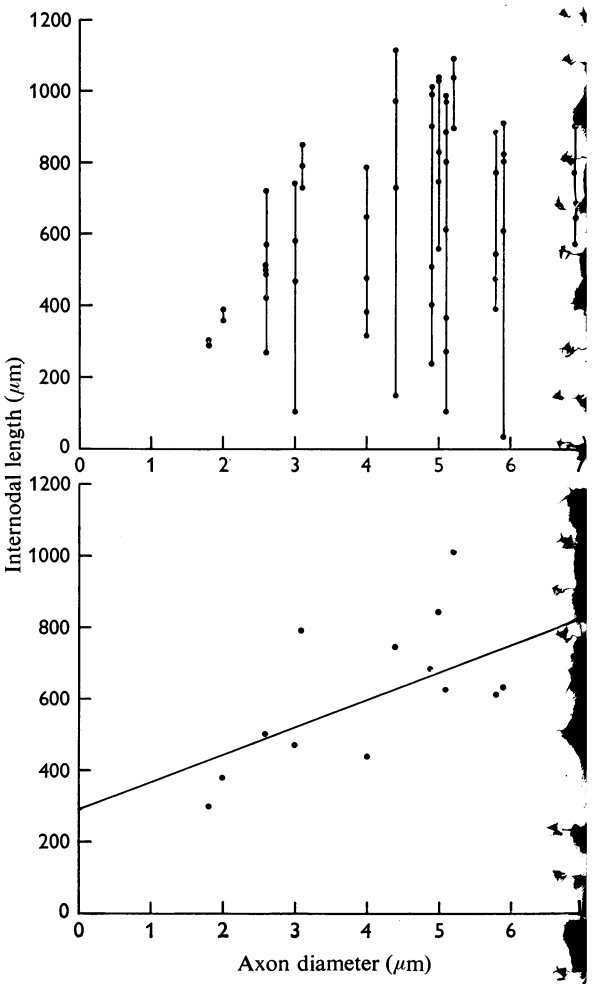

Fig. 6

Figs. 5, 6. *Upper graphs*: internodal lengths (μm) and axon diameters (μm) of remyelinating fibres from the sciatic nerve of chickens at 85 d (Fig. 5) and 150 d (Fig. 6) after nerve crush. *Lower graphs:* relationship between *mean* internodal lengths and axon diameters from the same nerve fibres.

Table 2. *Statistical data derived from results constituting Figs.* 5 *and* 6

Interval after crushing	Regression coefficient	Correlation coefficient	*y*-axis intercepts
Normal animal	1·46	0·90	188
85 d	0·60	0·80	72
150 d	0·41	0·71	292

Internodal lengths in chicken nerves re-innervated after crushing

The left sciatic nerves of two adult chickens were crushed and the nerves examined 85 and 150 d respectively after crushing. External diameters, axon diameters and internodal lengths were measured in teased fibres from the regenerating nerves. Results were recorded as in the previous section.

Eighty-five days after crushing (Fig. 5) axons are still of relatively small diameter, but already some indications of a positive relationship between mean internodal

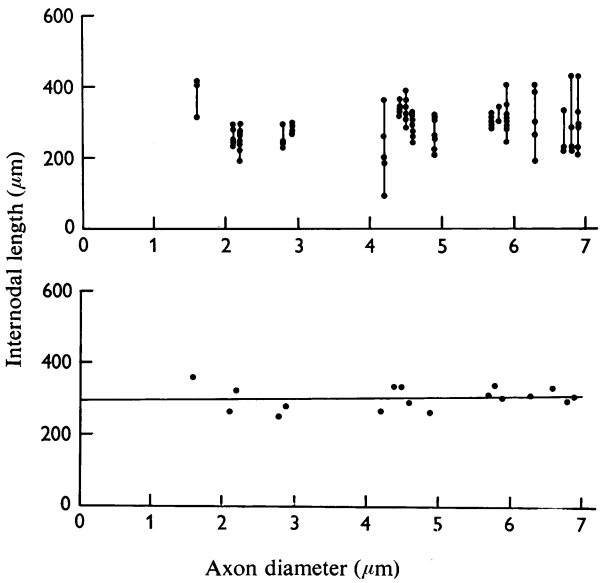

Fig. 7

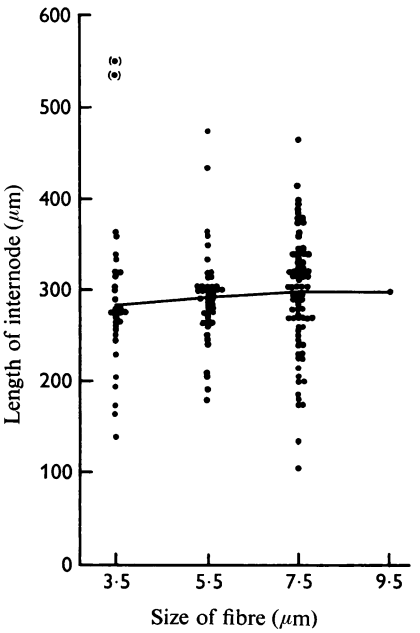

Fig. 8

Fig. 7. *Upper graph*: Internodal lengths (μm) and axon diameters (μm) of sciatic nerve fibres of rats remyelinating 165 d after intraneural injection of diphtheria toxin. *Lower graph*: Relationship between *mean* internodal lengths and axon diameters from the same fibres.

Fig. 8. Graph showing lengths of internodes in regenerated rat nerve fibres, 30 d to 17 weeks after crushing of nerve. The line connects calculated means. From Hiscoe (1947).

length along a fibre and axon diameter can be distinguished. One hundred and fifty days after crushing (Fig. 6) the full spectrum of axonal diameters appears to have been reached (see graph of normal fibres: Fig. 1). The scatter of internodal length along fibres is very wide, but there is still evidence of a linear relationship between internodal length and axon diameter. The range of internodal lengths is similar to that found in the fibres regenerating after a purely demyelinating lesion (Figs. 2–4). The smallest axons, between 1 and 2 μm diameter, have segments of *c.* 200 μm and the internodal lengths of the largest axons reaching about 1000 μm. Table 1 shows regression and correlation coefficients, and *y*-axis intercepts for these remyelinating fibres.

Internodal lengths in rats on recovery from demyelination caused by diphtheria toxin

Two adult rats were injected intraneurally with $2{\cdot}5 \times 10^{-2}$ L^+ diphtheria toxin. Both animals were killed 165 d after injection.

Measurements of external diameter, axon diameter and internodal length were made from the injected nerve. Results from both animals were combined and the results recorded (Fig. 7) as in the previous sections.

The graphs show that uniformly short internodes having a mean length of *c.* 300 μm are found in fibres remyelinating after a purely demyelinating lesion. For comparison, Hiscoe's (1947) results with remyelination following nerve crush in rats are shown (Fig. 8). Clearly the pattern of internodal lengths in this species is similar in both types of regeneration.

DISCUSSION

Regeneration of internodes in chickens. The pattern of new formation of internodes following a purely demyelinating lesion and after a nerve crush in chickens differs from that found by others after nerve crush in mammals. Thus, instead of new segments of fairly uniformly short length, the remyelinating internodes showed a tendency to vary in length directly as the calibre of the axons, so that the smallest axons (1–2 μm) had internodal lengths of *c.* 200 μm, while segments of the largest axons might reach 1000 μm or more.

Since the pattern of recovery of internodal segments was the same after both types of degeneration, it is concluded that the diameter of the axons within the remyelinating fibre is not the main factor affecting internodal length.

Regeneration of internodes in rats. Internodes of fairly uniformly short length were found in nerve fibres recovering from a demyelinating lesion. The internodal length–axon diameter relationship closely resembles that found by other workers in the rat and in other mammals after nerve crush. This supports the previous conclusion from chicken nerve that the diameter of the axons within regenerating Schwann cells is not the main factor affecting the ultimate pattern of internodal lengths.

These results also indicate that a species difference may exist in the mechanism of selection of cells for myelination, since there is no alteration in the pattern of internodal lengths once myelination is under way.

It is important to consider the possible implications of this unsuspected species difference in this context, and in order to seek some explanation for this difference it is necessary to retrace the events that occur prior to remyelination.

Excessive numbers of Schwann cells are produced by proliferation within degenerating fibres both after crushing and after diphtheria toxin. These are considerably more than are ultimately required to furnish the regenerating fibres with new internodes, since only one Schwann cell is ever associated with a single internode in these species. The initial stimulus to Schwann cell proliferation would seem, from our own experience, to stem from loss of the *specialized contact* of the cell with the axon. Proliferation in Wallerian degeneration does not cease until the spacing between Schwann nuclei within the tube is less than 100 μm, and usually in the region of 20 μm (unpublished observations in chickens and rats). Nuclear spacing tends to be

less in large fibres, possibly because of the greater capacity of the tube, than in small fibres, and also tends to be greater after selective myelin loss, but is rarely more than 100 μm. From this closely packed chain of cells a small number begin to myelinate and at the same time elongate, pushing out their non-myelinating fellows, until contact is made with another similarly determined cell. The final pattern of internodal lengths may thus ultimately be controlled by the mechanism that determines which Schwann cells initiate myelinogenesis and which shall disappear. The nature of such a choice is unknown.

The concept of the initial or basic length of the Schwann cell, first put forward by Young (1950), while relevant to the development of normal myelin internodes, has to be modified where repair mechanisms are considered, at least in chickens. He had observed that the internodes of young rabbits were about 250–300 μm; and this same value of internodal length reappeared in remyelinating fibres and also in the smallest fibres of adult animals. Young considered that there was some significance in this particular length; he proposed that it might be determined by physical factors involving the effects of surface tension upon myelin sheaths which he envisaged as behaving as cylinders of liquid (Young, 1945).

It now appears, however, that the segmentation of the myelin sheath may be largely determined by the spacing of the Schwann cells. It thus seems more relevant to consider that this minimum figure for internodal lengths, produced from a chain of cells that are very much more closely packed, is an expression of the spacing of the cells which first receive the stimulus to synthesize the membrane complex which constitutes the myelin sheath. This may well be largely a random selection of cells around a mean separation distance of 250 μm. The scatter around the mean in regenerated internodes after nerve crush in the rat appears to be greater than normal (Hiscoe, 1947) suggesting that the factors introducing variation in this process after injury have a lesser influence in normal development.

In chickens, however, the difference between the normal mechanism of internode cell selection and that following repair is even more marked. Measurements of fibres from sciatic and tibial nerves of young chicks at about the time of hatching have shown that their internodal lengths, visible with the light microscope, are 200–300 μm (J. M. Jacobs, 1964, unpublished observations). Thus, at this stage the Schwann cells appear to have the normal basic length found in the nerves of young mammals. However, during remyelination following both Wallerian degeneration and diphtheritic demyelination, the internodal lengths, particularly in the larger fibres, may attain 1000 μm or more. Thus, not only is the concept of a basic length of Schwann cell no longer tenable, but another factor is interpolated into this mechanism of cell choice in the chicken that does not apparently operate in the mammals so far studied.

SUMMARY

Internodal lengths have been measured in peripheral nerves of adult chickens and rats on recovery from nerve crush and from selective demyelination caused by locally inoculated diphtheria toxin.

In the rat the remyelinated internodes are of the same order of length after both processes and are independent of axon diameter. In the chicken, however, internodal

lengths tend to be related to axon diameter both after selective demyelination with preservation of the normal spectrum of axon diameters and following regeneration after a nerve crush. The regression coefficient of internodal length on axon diameter in this species is one-half to one-third that of the normal nerve. These observations are discussed in the light of the concept of the basic length of the Schwann cell, and the factors governing the selection of cells for myelination.

The work was carried out while Mrs J. M. Jacobs was holding a grant from the 'Action for the Crippled Child', which is gratefully acknowledged.

REFERENCES

ALLT, G. (1969). Ultrastructural features of the immature peripheral nerve. *J. Anat.* **105**, 283–293.

AITKEN, J. T., SHARMAN, M. & YOUNG, J. Z. (1947). Maturation of regenerating nerve fibres with various peripheral connections. *J. Anat.* **81**, 1–22.

AITKEN, J. T. & THOMAS, P. K. (1962). Retrograde changes in fibre size following nerve section. *J. Anat.* **96**, 121–129.

BAKER, J. R. (1960). *Cytological Technique*. London: Methuen and Co.

DUNCAN, D. (1934). A relation between axon cylinder and myelination determined by measurement of myelinated spinal root fibres. *J. comp. Neurol.* **60**, 437–472.

FULLERTON, P. M., GILLIATT, R. W., LASCELLES, R. G. & MORGAN-HUGHES, J. A. (1965). The relation between fibre diameter and internodal length in chronic neuropathy. *J. Physiol., Lond.* **178**, 26–28P.

GAMBLE, H. J. (1966). Further electron microscope studies of human foetal peripheral nerves. *J. Anat.* **100**, 487–502.

HILLARP, N. A. & OLIVECRONA, H. (1946). The role played by the axon and the Schwann cell in the degree of myelination of the peripheral nerve fibre. *Acta anat.* **2**, 17–32.

HISCOE, H. B. (1947). The distribution of nodes and incisures in normal and regenerated nerve fibres. *Anat. Rec.* **99**, 447–475.

JACOBS, J. M. (1967). Experimental diphtheritic neuropathy in the rat. *Br. J. exp. Path.* **48**, 204–216.

JACOBS, J. M., CAVANAGH, J. B. & MELLICK, R. S. (1966). Intraneural injection of diphtheria toxin. *Br. J. exp. Path.* **47**, 507–517.

MATHESON, D. F. (1968). Studies of the amino-acid incorporation into peripheral nerves. p. 91, Ph.D. Thesis, University of London.

QUILLIAM, T. (1958). Growth changes in sensory nerve fibre aggregates undergoing remyelination. *J. Anat.* **92**, 383–398.

SANDERS, F. K. & YOUNG, J. Z. (1944). The role of the peripheral stump in the control of fibre diameter in regenerating nerves. *J. Physiol., Lond.* **103**, 119–136.

SIMPSON, S. A. & YOUNG, J. Z. (1945). Regeneration of fibre diameter after cross-unions of visceral and somatic nerves. *J. Anat.* **79**, 48–65.

VIZOSO, A. D. & YOUNG, J. Z. (1948). Internode length and fibre diameter in developing and regenerating nerves. *J. Anat.* **82**, 110–134.

WENDELL-SMITH, C. P. & WILLIAMS, P. L. (1959). The use of teased preparations and frozen sections in quantitative studies of mammalian peripheral nerves. *Q. Jl microsc. Sci.* **100**, 499–508.

WILLIAMS, P. L. (1959). Sections of fresh mammalian nerve trunks for quantitative studies: Rapid freezing technique. *Q. Jl microsc. Sci.* **100**, 425–431.

WILLIAMS, P. L. & WENDELL-SMITH, C. P. (1958). Variations in axonal diameters of myelinated nerve fibres: Apparent or real? *Nature, Lond.* **181**, 125–126.

YOUNG, J. Z. (1945). The history of the shape of a nerve fibre. In *Essays on Growth and Form*. Oxford: Clarendon Press.

YOUNG, J. Z. (1950). *Genetic Neurology* (ed. P. Weiss), University of Chicago Press.